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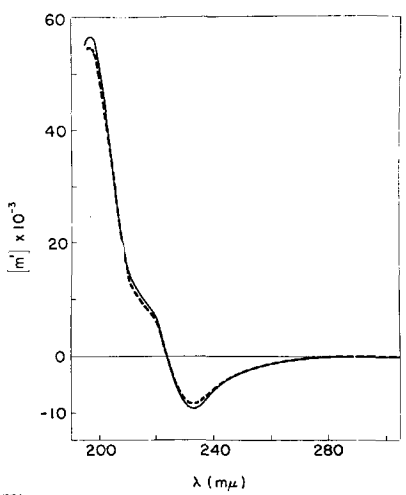
Optical rotatory dispersion and circular dichroism of phospholipase A_2 and its zymogen from porcine pancreas

Phospholipase A, isolated in a pure form from porcine pancreas, acts on 3-*sn*-phosphoglycerides with specific hydrolysis of fatty acid ester bonds at the glycerol C-2 position[1]. The protein has a molecular weight of 13 800 ± 500 and appears to consist of a single polypeptide chain with alanine and cystine as C- and N-terminals. A single-chain precursor of phospholipase A_2 which has a molecular weight of about 15 000 is converted to the catalytically active form by trypsin[2], which releases a heptapeptide, Glu–Glu–Gly–Ile–Ser–Ser–Arg, from the N-terminal end. In this peptide as well as in the zymogen itself the N-terminal glutamic acid has no free α-NH_2 group. In order to obtain information on the relative conformation of these proteins, the optical rotatory dispersion and circular dichroic properties of prephospholipase A_2 and phospholipase A_2 have been compared.

Prephospholipase A_2 and phospholipase A_2 were prepared from pancreatic tissue as previously described[1,2], and kept in lyophilized form until use. As judged by polyacrylamide-gel electrophoresis both preparations were single components. Urea (Baker Chemical Co., Phillipsburg, N.J.) was recrystallized from 50% ethanol. Guanidine–HCl was prepared from guanidine carbonate (Matheson Co, Joliet, Ill.) and was recrystallized from ethanol. A Cary Model 6001 spectropolarimeter equipped with a circular dichroism attachment was used to record the optical rotatory dispersion and circular dichroic spectra. All measurements were made at 27° using a 0.1-ml path-length cell (Pyrocell, Orangeburg, N.J.) filled with solutions containing 1–2 mg of protein. The value of molar ellipticity $[\theta]$ was obtained from the relation $[\theta] = (\theta/10) \cdot (M/lc)$ where θ was observed ellipticity, M (mean residue weight) was 112, l was



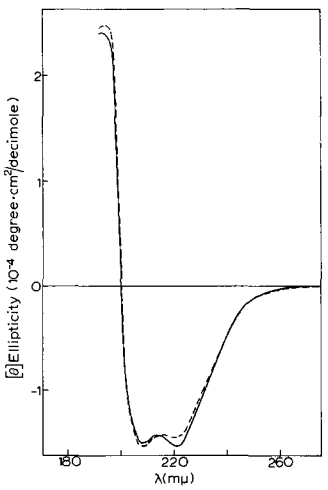


Fig. 1. Optical rotatory dispersion patterns of phospholipase A_2 (———) and prephospholipase A_2 (— — —), in phosphate buffer (pH 7.4) at 27° and protein concentrations of 1 to 2 mg/ml.

Fig. 2. Circular dichroic spectra of phospholipase A_2 (———) and prephospholipase A_2 (— — —) in phosphate buffer (pH 7.4) at 27° and protein concentrations of 1 to 2 mg/ml.

cell path length in cm and c was concentration of solute in g/ml. Protein content was determined by the method of LOWRY *et al.*[3].

As demonstrated in Fig. 1, both prephospholipase A_2 and phospholipase A_2 gave very similar optical rotatory dispersion patterns with features typical of an α-helix, namely a negative trough with a minimum at 233 mμ, a cross-over point at 224 mμ, a shoulder between 210 and 215 mμ, and a positive Cotton effect with a maximum at 198 mμ. The rotatory parameters of prephospholipase A_2 were slightly but consistently lower than those with phospholipase A_2. The circular dichroic spectra (Fig. 2), in agreement with the optical rotatory dispersion studies, were compatible with the presence of an α-helix and were characterized by two negative bands with extrema at 222 and 209 mμ and a positive peak with a maximum at 194 mμ. As indicated in Fig. 2 the band positions were identical in both products. Spectral differences between phospholipase A_2 and its precursor were limited. Values of 50 and 55% α-helix for prephospholipase A_2 and phospholipase A_2 were calculated using ellipticity values of the 222-mμ band. In both prephospholipase A_2 and phospholipase A_2 an increase of pH of the medium to 9 to 11 brought about an accentuation of the 208-mμ band, without changing the typical α-helical pattern. The spectra of both prephospholipase A_2 and phospholipase A were not affected in 8 M solutions of urea, while only limited effects indicated by a slight reduction of the molar ellipticity value of the 222-mμ band were observed in media containing 7 M guanidine–HCl (Fig. 3). The spectra were not

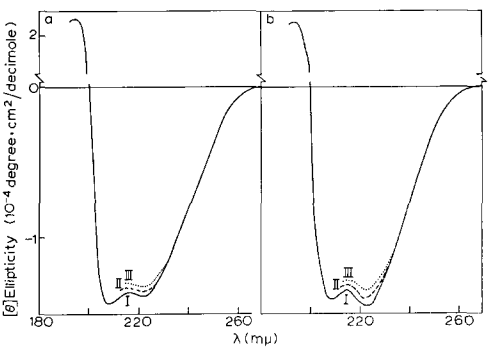


Fig. 3. Circular dichroic spectra of phospholipase A_2 (b) and prephospholipase A_2 (a) before (I) and after addition of 8 M urea (II) and 7 M guanidine–HCl (III). All experiments were carried out at pH 7.4 and 27°.

affected by prolonging the contact of the dissociating agent with the enzyme preparation up to 4 days. This observation substantiates the finding that the enzymatic activity of phospholipase A_2 was not impaired after exposure to these agents[4]. Reduction of both prephospholipase A_2 and phospholipase A_2 caused a complete inactivation of the enzyme and a disappearance of all electronic transitions indicative of a conversion toward a disordered structure.

In summary, the present spectral studies indicate that both the zymogen and the active form of phospholipase A_2 from porcine pancreas have a high content in α-helix, the non-helical portion of the molecule probably representing a disordered structure. The activation of prephospholipase A appears to give some conformational changes compatible with a slight increase of α-helical content in the active form of

the enzyme. Whether this represents a true increase in helical content or is simply the consequence of the splitting off of the N-terminal heptapeptide cannot be ascertained by the present studies. Both proteins resist spectral changes in the presence of high concentrations of urea or guanidine–HCl, a behaviour probably related to the high content of disulphide bridges in phospholipase A_2 and its zymogen.

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Viscosity and hydrogen ion equilibrium studies of the effects of organic solvents on the conformation of bovine serum albumin in aqueous solutions

The conformation of bovine serum albumin undergoes reversible changes in the pH region below 4.3 (refs. 1 and 2). The changes are caused by electrostatic forces[3] and are mainly in the form of expansion. In this communication we report the effect of organic solvents on the conformation of bovine serum albumin in acidic aqueous solutions.

Viscosity measurements were carried out at 25.00 ± 0.05°, using a Cannon–Ubbelohde No. 50 viscometer described in the previous paper[4]. The flow time for water with this viscometer was 227.2 sec. The precision was about 0.1 sec; occasionally, larger deviations were observed. The kinetic energy correction was negligible with this viscometer.

Titration was performed with a Radiometer titrator type TTTIC connected to a scale expander type pH A630T. The temperature was kept constant at 25.00 ± 0.05°.